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International application number: PCT/US04/018962

International filing date: 14 June 2004 (14.06.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/478,205  
Filing date: 13 June 2003 (13.06.2003)

Date of receipt at the International Bureau: 30 August 2004 (30.08.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
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APPLICATION NUMBER: 60/478,205  
FILING DATE: *June 13, 2003*  
RELATED PCT APPLICATION NUMBER: PCT/US04/18962

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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EV146603892US

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## TITLE OF THE INVENTION (500 characters max)

EXPRESSION VECTORS, VACCINES AND INNUMOTHERAPEUTICS INCLUDING MODIFICATIONS OF THE SAME WITH IgE SIGNAL PEPTIDE AND METHODS FOR USING THE SAME

## CORRESPONDENCE ADDRESS

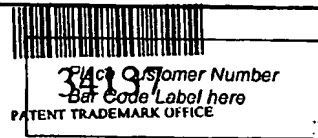
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## ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages

62

☐ CD(s), Number

☒ Drawing(s) Number of Sheets

7

☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

SIGNATURE

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Date

June 13, 2003

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REGISTRATION NO.  
(if appropriate)

33,229

Docket Number:

UPAP0022-001

TELEPHONE

215-665-5592

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**EXPRESSION VECTORS, VACCINES AND IMMUNOTHERAPEUTICS  
INCLUDING MODIFICATIONS OF THE SAME WITH IgE SIGNAL PEPTIDE  
AND METHODS FOR USING THE SAME**

**FIELD OF THE INVENTION**

The present invention relates to improved vaccines, improved methods for prophylactically and/or therapeutically immunizing individuals against immunogens, and to improved immunotherapeutic compositions and improved immunotherapy methods.

**5 BACKGROUND OF THE INVENTION**

Immunotherapy refers to modulating a person's immune responses to impart a desirable therapeutic effect. Immunotherapeutics refer to those compositions which, when administered to an individual, modulate the individual's immune system sufficient to ultimately decrease symptoms which are associated with undesirable immune responses or to  
10 ultimately alleviate symptoms by increasing desirable immune responses. In some cases, immunotherapy is part of a vaccination protocol in which the individual is administered a vaccine that exposes the individual to an immunogen against which the individual generates an immune response. In such cases, the immunotherapeutic increases the immune response and/or selectively enhances a portion of the immune response (such as the cellular arm or the

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humoral arm) which is desirable to treat or prevent the particular condition, infection or disease.

Vaccines are useful to immunize individuals against target antigens such as allergens, pathogen antigens or antigens associated with cells involved in human diseases. Antigens associated with cells involved in human diseases include cancer-associated tumor antigens and antigens associated with cells involved in autoimmune diseases.

In designing such vaccines, it has been recognized that vaccines that produce the target antigen in cells of the vaccinated individual are effective in inducing the cellular arm of the immune system. Specifically, live attenuated vaccines, recombinant vaccines which use avirulent vectors, and DNA vaccines each lead to the production of antigens in the cell of the vaccinated individual which results in induction of the cellular arm of the immune system. On the other hand, killed or inactivated vaccines, and sub-unit vaccines which comprise only proteins do not induce good cellular immune responses although they do induce a humoral response.

A cellular immune response is often necessary to provide protection against pathogen infection and to provide effective immune-mediated therapy for treatment of pathogen infection, cancer or autoimmune diseases. Accordingly, vaccines that produce the target antigen in cells of the vaccinated individual such as live attenuated vaccines, recombinant vaccines that use avirulent vectors and DNA vaccines are often preferred.

While such vaccines are often effective to immunize individuals prophylactically or therapeutically against pathogen infection or human diseases, there is a need for improved vaccines. There is a need for compositions and methods that produce an enhanced immune response.

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Likewise, while some immunotherapeutics are useful to modulate immune response in a patient there remains a need for improved immunotherapeutic compositions and methods.

Gene therapy refers to the delivery of genes to an individual who is in need of or can otherwise benefit by the protein encoded by the protein. Numerous strategies have been developed to deliver proteins for which the individual does not have a corresponding gene that produces sufficient and/or fully functional protein. Thus the gene therapy compensates for the lack of sufficient fully functioning endogenous protein. In some gene therapy strategy, the patient is provided with a therapeutically effective protein using constructs designed to produce a therapeutically effective amount of the protein. The gene therapy provides an alternative method for delivering protein therapeutics. There remains a need for improved gene therapy compositions and methods.

In addition to direct administration of nucleic acid molecules to individuals, proteins are often delivered. Production of such proteins by recombinant methods is often the most efficient way to manufacturing them. There remains a need for improved protein manufacturing compositions and methods.

#### SUMMARY OF THE INVENTION

The present invention relates to nucleic acid molecules that comprise a nucleic acid sequence that encode fusion proteins that consists of an IgE signal peptide linked to non-IgE protein sequences wherein the IgE signal peptide and the non-IgE protein sequences are derived from the same animal species.

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The present invention relates to in vitro host cell cultures comprising expression vectors operable in such host cells that comprise a nucleic acid sequence that encode fusion proteins that consists of an IgE signal peptide linked to non-IgE protein sequences; to such nucleic acid molecules; and to host cells comprising such vectors.

5       The present invention relates to nucleic acid molecules that comprise nucleic acid sequences that encode fusion proteins that comprising an IgE signal peptide linked to non-IgE protein sequences operably linked to regulatory elements required for expression and nucleic acid sequences that encode an immunogen operably linked to regulatory elements required for expression.

10       The present invention relates to compositions that comprise nucleic acid molecules that comprises nucleic acid sequences that encode fusion proteins that comprising an IgE signal peptide linked to non-IgE protein sequences and nucleic acid molecules that comprise nucleic acid sequences that encode an immunogen, wherein the nucleic acid molecules that comprises nucleic acid sequences that encode the fusion protein are not identical to the  
15       nucleic acid molecules that comprise nucleic acid sequences that encode the immunogen.

The present invention relates to isolated fusion proteins that comprise an IgE signal peptide linked to non-IgE protein sequences.

The present invention relates to methods of modulating an immune response in an individual that comprise administering to an individual a composition that comprises a  
20       nucleic acid molecule comprising nucleic acid sequences that encode fusion proteins that comprising an IgE signal peptide linked to an immunomodulatory protein.

The present invention relates to methods of inducing an immune response against an immunogen in an individual that comprise administering to an individual nucleic acid

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molecules that comprise a nucleic acid sequence that encodes a fusion protein that comprises an IgE signal peptide linked to an immunomodulatory protein and a nucleic acid sequence that encodes an immunogen. The various coding sequences for the different proteins may be on the same nucleic acid molecule and/or different nucleic acid molecule.

5           The present invention relates to recombinant vaccines that comprise a nucleic acid molecule that comprises a nucleic acid sequence that encodes an immunogen and a nucleic acid sequence that encodes a fusion protein comprising an IgE signal sequence linked to an immunomodulatory protein; and to methods of immunizing an individual against an immunogen comprising administering to an individual such recombinant vaccines.

10           The present invention relates to live attenuated pathogens that comprise a nucleic acid molecule that comprises a nucleic acid sequence that encodes a fusion protein comprising an IgE signal sequence linked to an immunomodulating protein; and to methods of immunizing an individual against a pathogen comprising administering to an individual such live attenuated pathogens.

15           The present invention relates to nucleic acid molecules that comprise a nucleic acid sequence that encodes a fusion protein that comprises an IgE signal peptide linked to IL-15 protein sequences; to vectors comprising such nucleic acid molecules; and to host cells comprising such vectors.

20           The present invention relates to fusion proteins that comprise an IgE signal peptide linked to IL-15 protein sequences.

          The present invention relates to compositions that comprise a nucleic acid molecule that comprises a nucleic acid sequence that encodes a fusion protein that comprises an IgE signal peptide linked to IL-15 protein sequences, and a nucleic acid molecule that comprises



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a nucleic acid sequence that encodes an immunogen. Optionally, a nucleic acid sequence that encodes CD40L may be present in nucleic acid molecules that comprise a nucleic acid sequence that encodes the fusion protein and/or the immunogen or in a separate nucleic acid molecule.

5       The present invention relates to methods of modulating an immune response in an individual comprising administering to an individual a composition that comprises one or more nucleic acid molecules that comprise a nucleic acid sequence that encodes a fusion protein that comprise an IgE signal peptide linked to IL-15 protein, and optionally a nucleic acid sequence that encodes CD40L. The various nucleic acid sequences that encode the  
10 various different proteins may be on the same nucleic acid molecule and/or different nucleic acid molecules or both.

      The present invention relates to methods of inducing an immune response against an immunogen in an individual that comprises administering to an individual a composition that comprises one or more nucleic acid molecules that comprises a nucleic acid sequence that  
15 encodes a fusion protein that comprises an IgE signal peptide linked to IL-15 protein sequences, a nucleic acid sequence that encodes an immunogen and optionally, a nucleic acid sequence that encodes CD40L. The various nucleic acid sequences that encode the various different proteins may be on the same nucleic acid molecule and/or different nucleic acid molecules or combinations thereof.

20

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1, Figure 2, Figure 3 panels A-C, Figure 4 panels A-B, Figure 5 and Figure 6 refer to the disclosure set forth in Example 1.

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Figure 7 refers to data from the disclosure set forth in Example 2.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

### Definitions

5 As used herein the term "target protein" is meant to refer to peptides and protein encoded by gene constructs of the present invention that act as target proteins for an immune response. The terms "target protein" and "immunogen" are used interchangeably and refer to a protein against which an immune response can be elicited. The target protein is an immunogenic protein that shares at least an epitope with a protein from the pathogen or  
10 undesirable cell-type such as a cancer cell or a cell involved in autoimmune disease against which an immune response is desired. The immune response directed against the target protein will protect the individual against and/or treat the individual for the specific infection or disease with which the target protein is associated.

As used herein, the term "genetic construct" refers to the DNA or RNA molecules that  
15 comprise a nucleotide sequence that encodes a target protein or immunomodulating protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered.

As used herein, the term "expressible form" refers to gene constructs that contain the  
20 necessary regulatory elements operable linked to a coding sequence that encodes a target protein or an immunomodulating protein, such that when present in the cell of the individual, the coding sequence will be expressed.

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As used herein, the term "sharing an epitope" refers to proteins that comprise at least one epitope that is identical to or substantially similar to an epitope of another protein.

As used herein, the term "substantially similar epitope" is meant to refer to an epitope that has a structure that is not identical to an epitope of a protein but nonetheless invokes a cellular or humoral immune response which cross reacts to that protein.

As used herein, the term "intracellular pathogen" is meant to refer to a virus or pathogenic organism that, at least part of its reproductive or life cycle, exists within a host cell and therein produces or causes to be produced, pathogen proteins.

As used herein, the term "hyperproliferative diseases" is meant to refer to those diseases and disorders characterized by hyperproliferation of cells.

As used herein, the term "hyperproliferative-associated protein" is meant to refer to proteins that are associated with a hyperproliferative disease.

As used herein the term "immunomodulating protein" refers to a protein that modulates the immune system of a person to whom the immunomodulating protein is delivered. Examples of immunomodulatory proteins include: IL-15, CD40L, TRAIL; TRAIL<sub>recDRC5</sub>, TRAIL-R2, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, F46181 or MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, CD30, CD153 (CD30L), Fos, c-jun, Sp-1, Ap1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, I $\kappa$ B, NIK, SAP K, SAP1, JNK2, JNK1B2, JNK1B1, JNK2B2, JNK2B1, JNK1A2, JNK2A1, JNK3A1, JNK3A2, NF-kappa-B2, p49 splice form, NF-kappa-B2, p100 splice form, NF-kappa-B2, p105 splice form, NF-kappa-B 50K chain precursor, NFkB p50, human IL-1  $\alpha$ , human IL-2, human IL-4, murine IL-4, human IL-5, human IL-10, human IL-15, human IL-18, human TNF-  $\alpha$ , human TNF-  $\beta$ , human interleukin 12, MadCAM-1, NGF IL-7,

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VEGF, TNF-R, Fas, CD40L, IL-4, CSF, G-CSF, GM-CSF, M-CSF, LFA-3, ICAM-3, ICAM-2, ICAM-1, PECAM, P150.95, Mac-1, LFA-1, CD34, RANTES, IL-8, MIP-1 $\alpha$ , E-selecton, CD2, MCP-1, L-selecton, P-selecton, FLT, Apo-1, Fas, TNFR-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4(TRAIL), DR5, KILLER, TRAIL-R2, TRICK2,  
 5 DR6, ICE, VLA-1, CD86 (B7.2) and functional fragments thereof.

As used herein, "functional fragment" is meant to refer to a fragment of an immunomodulating protein that, when delivered in conjunction with an immunogen, provides an increased immune response compared to the immune that is induced when the immunogen is delivered without the fragment. Fragments are generally 10 or more amino  
 10 acids in length.

#### Overview

The invention arises from the discovery that fusion proteins that comprise the IgE signal peptide facilitate enhanced expression and are particularly, *inter alia*, useful in protein production, vaccines and gene therapeutics such as for the delivery of proteins such as  
 15 immunomodulating proteins. In some preferred embodiments, the invention provides vectors, vaccines and immunomodulatory compositions and methods comprising nucleic acid molecules that comprise nucleotide sequences that encode proteins including fusion proteins in which the IgE signal peptide is linked to human IL-15 coding sequences, preferably of IL-15 signal sequence, and more preferably free of the IL-15 Kozak region and untranslated  
 20 regions. In some preferred embodiments, the invention provides vectors, vaccines and immunomodulatory compositions and methods comprising nucleic acid molecules that comprise nucleotide sequences that encode fusion proteins which consist of: human IgE

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signal sequence linked to human IL-15 protein free of IL-15 signal sequence in combination with nucleotide sequences that encode human CD40L.

5 **Fusion proteins comprising and genetic constructs encoding IgE signal sequence linked to non-IgE protein**

Thus, one general aspect of the invention relates to fusion proteins comprising and genetic constructs encoding IgE signal sequence linked to non-IgE protein and the use of such constructs in expression vectors, vaccines and immunomodulatory compositions. Several different embodiments and forms are provided with respect to this aspect.

10 According to some embodiments, compositions are provided which comprise an isolated nucleic acid molecule comprising a nucleic acid sequence that encodes a fusion protein comprising an IgE signal sequence operable linked to non-IgE protein sequence.

The nature of the non-IgE protein is dependent upon the intended use of the constructs. For example, for gene therapy embodiments, the protein sequences would be  
15 those of a desired protein such as a protein for which patient lacks sufficient amounts of a functioning or fully functioning protein. Examples of this type of desired protein include enzymes such as DNase, growth factors such as growth hormone (human, bovine, porcine), clotting factors, insulin, dystrophin, and the like. The desired protein may also be one that when expressed in the patient provides a therapeutic benefit such as erythropoietin, IL-2,  
20 GM-CSF, TPA, and the like. In some embodiments, the non-IgE protein sequence is an immunogen. Such constructs are useful in vaccines in which expression of the immunogen is provided as a target for an immune response. In some embodiments, the non-IgE protein sequence is an immunomodulating protein. Such constructs are useful in vaccines in which expression of the immunogen is provided as a target for an immune response as well as  
25 immunomodulatory compositions in which the desired effect is to have the immune system of

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a patient, or a specific aspect of the immune system upregulated or downregulated depending upon the condition of the patient being treated. Immunomodulators that upregulate the immune system are useful to treat patients suffering immunosuppression or infectious diseases for example while those that downregulate the immune system are useful for  
5 example to treat autoimmune diseases, patients receiving organ transplants, tissue grafts or cell therapy for whom immune suppression is desirable. In some embodiment, the IgE signal sequence is linked to a non-IgE protein sequence for use in a system in which production of the IgE protein is desirable. In preferred embodiments, the IgE signal peptide is derived as from the same species of animal as the protein sequences to which it is linked. In preferred  
10 methods, the animal being administered such constructs is the same species as the animal from which the IgE signal peptide and protein sequence are derived. Such fusion proteins would be considered to be non-immunogenic.

In some embodiments, compositions that include a construct which comprise coding sequences of IgE signal linked to non-IgE protein sequence that are immunomodulating  
15 protein may also include on the same nucleic acid molecule or a different nucleic acid molecule, a nucleic acid sequence that encodes an immunogen. Generally, immunogens, which are discussed below, may be any immunogenic protein including allergens, pathogen antigens, cancer-associated antigens or antigens linked to cells associated with autoimmune diseases. In preferred embodiments, the immunogen is a pathogen antigen, most preferably a  
20 pathogen selected from the group consisting of HIV, HSV, HCV, and WNV.

As noted above, the non-IgE protein sequence is preferably an IL-15 protein, more preferably an IL-15 protein free of IL-15 signal sequence, more preferably more preferably

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an IL-15 protein free of IL-15 signal sequence, free of IL-15 Kozak region and free of IL-15 untranslated sequences.

In preferred embodiments, the composition further comprises a nucleotide sequence that encodes CD40L. This nucleotide sequence may be included on the same nucleic acid molecule as the fusion protein or a different molecule. The CD40L may be included in vaccine compositions that include coding sequences for immunogens, yielding improved vaccines. In other embodiments, the CD40L may be included in immunomodulatory compositions that do not include coding sequences for immunogens, yielding improved immunomodulatory compositions.

In some preferred embodiments, nucleic acid constructs are plasmids. In some preferred embodiments, the nucleic acid molecule is incorporated in a viral vector such as vaccinia, adenovirus, adenovirus associated virus, retrovirus, or any other acceptable viral vector useful as a vaccine or gene therapy vector.

Genetic constructs comprising IgE signal sequences linked to non-IgE protein sequence that are immunomodulating proteins may be incorporated directly into live attenuated pathogens according to some aspect of the invention. Examples of such pathogens useful as vaccines are set out below. In preferred embodiments, the immunomodulating protein is IL-15, more preferably IL-15 protein free of IL-15 signal sequence, more preferably IL-15 protein free of IL-15 signal sequence free of IL-15 Kozak region and free of IL-15 untranslated sequences. In some embodiments, such attenuated pathogens are further provided with a nucleotide sequence that encodes CD40L.

A fusion protein that comprise an IgE signal sequence operable linked to non-IgE protein sequences are also aspects of the invention. In some embodiments, the non-IgE

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protein sequence portion of the fusion protein is an enzyme. In some embodiments, the non-IgE protein sequence portion of the fusion protein is an immunogen. In some embodiments, the non-IgE protein sequence portion of the fusion protein is an immunomodulating protein. The preferred non-IgE protein sequence is IL-15 protein, most preferably free of IL-15 signal sequence.

#### Vaccines and immunomodulatory compositions

According to some embodiments of the invention, compositions of the invention comprise genetic constructs including coding sequences for immunogens and/or immunogenic proteins. Such compositions are delivered to an individual to modulate the activity of the individual's immune system and thereby enhance the immune response against the immunogen. When the nucleic acid molecules that encode an immunomodulatory protein are taken up by cells of the individual the nucleotide sequences that encode the immunomodulatory protein are expressed in the cells and the proteins are thereby delivered to the individual. Aspects of the invention provide methods of delivering the coding sequences of the proteins on a single nucleic acid molecule, in compositions comprising different nucleic acid molecules that encodes one or more of the various transcription factor or intermediate factors, as part of recombinant vaccines and as part of attenuated vaccines.

According to some aspects of the present invention, compositions and methods are provided which prophylactically and/or therapeutically immunize an individual against a pathogen or abnormal, disease-related cells. The vaccine may be any type of vaccine such as, a live attenuated vaccine, a cell vaccine, a recombinant vaccine or a nucleic acid or DNA vaccine.



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The present invention relates to compositions for delivering the immunomodulating proteins and methods of using the same.

The nucleic acid molecules may be delivered using any of several well known technologies including DNA injection (also referred to as DNA vaccination), recombinant  
5 vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia.

DNA vaccines are described in U.S. Patent Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, 5,676,594, and the priority applications cited therein, which are each incorporated herein by reference. In addition to the  
10 delivery protocols described in those applications, alternative methods of delivering DNA are described in US. Patent Nos. 4,945,050 and 5,036,006, which are both incorporated herein by reference.

Routes of administration include, but are not limited to, intramuscular, intranasally, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraocularly and oral  
15 as well as topically, transdermally, by inhalation or suppository or to mucosal tissue such as by lavage to vaginal, rectal, urethral, buccal and sublingual tissue. Preferred routes of administration include to mucosal tissue, intramuscular, intraperitoneal, intradermal and subcutaneous injection. Genetic constructs may be administered by means including, but not limited to, traditional syringes, needleless injection devices, or "microprojectile bombardment  
20 gene guns".

When taken up by a cell, the genetic construct(s) may remain present in the cell as a functioning extrachromosomal molecule and/or integrate into the cell's chromosomal DNA. DNA may be introduced into cells where it remains as separate genetic material in the form

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of a plasmid or plasmids. Alternatively, linear DNA that can integrate into the chromosome may be introduced into the cell. When introducing DNA into the cell, reagents that promote DNA integration into chromosomes may be added. DNA sequences that are useful to promote integration may also be included in the DNA molecule. Alternatively, RNA may be administered to the cell. It is also contemplated to provide the genetic construct as a linear minichromosome including a centromere, telomeres and an origin of replication. Gene constructs may remain part of the genetic material in attenuated live microorganisms or recombinant microbial vectors which live in cells. Gene constructs may be part of genomes of recombinant viral vaccines where the genetic material either integrates into the chromosome of the cell or remains extrachromosomal. Genetic constructs include regulatory elements necessary for gene expression of a nucleic acid molecule. The elements include: a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers are often required for gene expression of the sequence that encodes the target protein or the immunomodulating protein. It is necessary that these elements be operably linked to the sequence that encodes the desired proteins and that the regulatory elements are operably in the individual to whom they are administered.

Initiation codons and stop codon are generally considered to be part of a nucleotide sequence that encodes the desired protein. However, it is necessary that these elements are functional in the individual to whom the gene construct is administered. The initiation and termination codons must be in frame with the coding sequence.

Promoters and polyadenylation signals used must be functional within the cells of the individual.

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Examples of promoters useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to promoters from Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, 5 Moloney virus, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human Myosin, human Hemoglobin, human muscle creatine and human metallothionein.

10 Examples of polyadenylation signals useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to SV40 polyadenylation signals and LTR polyadenylation signals. In particular, the SV40 polyadenylation signal that is in pCEP4 plasmid (Invitrogen, San Diego CA), referred to as the SV40 polyadenylation signal, is used.

15 In addition to the regulatory elements required for DNA expression, other elements may also be included in the DNA molecule. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

20 Genetic constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. Plasmids pVAX1, pCEP4 and pREP4 from Invitrogen (San Diego, CA) contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region which produces high copy episomal replication without integration.

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In some preferred embodiments related to immunization applications, nucleic acid molecule(s) are delivered which include nucleotide sequences that encode a target protein, the immunomodulating protein and, additionally, genes for proteins which further enhance the immune response against such target proteins. Examples of such genes are those which  
5 encode other cytokines and lymphokines such as alpha-interferon, gamma-interferon, platelet derived growth factor (PDGF), TNF, GM-CSF, epidermal growth factor (EGF), IL- 1, IL-2, IL-4, IL-6, IL-10, IL-12 and IL- 15 including IL-15 having the signal sequence deleted and optionally including the signal peptide from IgE.

An additional element may be added which serves as a target for cell destruction if it  
10 is desirable to eliminate cells receiving the genetic construct for any reason. A herpes thymidine kinase (tk) gene in an expressible form can be included in the genetic construct. The drug gancyclovir can be administered to the individual and that drug will cause the selective killing of any cell producing tk, thus, providing the means for the selective destruction of cells with the genetic construct.

15 In order to maximize protein production, regulatory sequences may be selected which are well suited for gene expression in the cells the construct is administered into. Moreover, codons may be selected which are most efficiently transcribed in the cell. One having ordinary skill in the art can produce DNA constructs that are functional in the cells.

20 One method of the present invention comprises the steps of administering nucleic acid molecules intramuscularly, intranasally, intraperitoneally, subcutaneously, intradermally, or topically or by lavage to mucosal tissue selected from the group consisting of inhalation, vaginal, rectal, urethral, buccal and sublingual.

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In some embodiments, the nucleic acid molecule is delivered to the cells in conjunction with administration of a polynucleotide function enhancer or a genetic vaccine facilitator agent. Polynucleotide function enhancers are described in U.S. Serial Number 5,593,972, 5,962,428 and International Application Serial Number PCT/US94/00899 filed  
5 January 26, 1994, which are each incorporated herein by reference. Genetic vaccine facilitator agents are described in US. Serial Number 021,579 filed April 1, 1994, which is incorporated herein by reference. The co-agents that are administered in conjunction with nucleic acid molecules may be administered as a mixture with the nucleic acid molecule or administered separately simultaneously, before or after administration of nucleic acid  
10 molecules. In addition, other agents which may function transfecting agents and/or replicating agents and/or inflammatory agents and which may be co-administered with a GVF include growth factors, cytokines and lymphokines such as  $\alpha$ -interferon, gamma-interferon, GM-CSF, platelet derived growth factor (PDGF), TNF, epidermal growth factor (EGF), ILA, IL-2, IL-4, IL-6, IL-10, IL-12 and IL-15 as well as fibroblast growth factor, surface active  
15 agents such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl Lipid A (WL), muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the genetic construct. In some embodiments, an immunomodulating protein may be used as a GVF. In some embodiments, the nucleic acid molecule is provided in  
20 association with PLG to enhance delivery/uptake.

The pharmaceutical compositions according to the present invention comprise about 1 nanogram to about 2000 micrograms of DNA. In some preferred embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram

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to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 100 to about 200 microgram DNA.

The pharmaceutical compositions according to the present invention are formulated according to the mode of administration to be used. In cases where pharmaceutical compositions are injectable pharmaceutical compositions, they are sterile, pyrogen free and particulate free. An isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. In some embodiments, a vasoconstriction agent is added to the formulation.

According to some embodiments of the invention, methods of inducing immune responses against an immunogen are provided by delivering compositions of the invention to an individual. The vaccine may be a live attenuated vaccine, a cell vaccine, a recombinant vaccine or a nucleic acid or DNA vaccine.

In addition to using expressible forms of immunomodulating protein coding sequence to improve genetic vaccines, the present invention relates to improved attenuated live vaccines and improved vaccines that use recombinant vectors to deliver foreign genes that

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encode antigens. Examples of attenuated live vaccines and those using recombinant vectors to deliver foreign antigens are described in U.S. Patent Nos.: 4,722,848; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,364; 5,462,734; 5,470,734; and 5,482,713, which are each incorporated herein by reference. Gene constructs are provided which include the nucleotide sequence that encodes an immunomodulating protein is operably linked to regulatory sequences that can function in the vaccine to effect expression. The gene constructs are incorporated in the attenuated live vaccines and recombinant vaccines to produce improved vaccines according to the invention.

10 The present invention provides an improved method of immunizing individuals that comprises the step of delivering gene constructs to the cells of individuals as part of vaccine compositions which include are provided which include DNA vaccines, attenuated live vaccines and recombinant vaccines. The gene constructs comprise a nucleotide sequence that encodes an immunomodulating protein and that is operably linked to regulatory sequences that can function in the vaccine to effect expression. The improved vaccines result in an enhanced cellular immune response.

### Immunogens

The present invention is useful to elicit enhanced immune responses against a target protein, i.e. proteins specifically associated with pathogens, allergens or the individual's own "abnormal" cells. The present invention is useful to immunize individuals against pathogenic agents and organisms such that an immune response against a pathogen protein provides protective immunity against the pathogen. The present invention is useful to combat hyperproliferative diseases and disorders such as cancer by eliciting an immune response

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against a target protein that is specifically associated with the hyperproliferative cells. The present invention is useful to combat autoimmune diseases and disorders by eliciting an immune response against a target protein that is specifically associated with cells involved in the autoimmune condition.

5       According to some aspects of the present invention, DNA or RNA that encodes a target protein and immunomodulating proteins is introduced into the cells of tissue of an individual where it is expressed, thus producing the encoded proteins. The DNA or RNA sequences encoding the target protein and one or both immunomodulating proteins are linked to regulatory elements necessary for expression in the cells of the individual. Regulatory  
10       elements for DNA expression include a promoter and a polyadenylation signal. In addition, other elements, such as a Kozak region, may also be included in the genetic construct.

In some embodiments, expressible forms of sequences that encode the target protein and expressible forms of sequences that encode both immunomodulating proteins are found on the same nucleic acid molecule that is delivered to the individual.

15       In some embodiments, expressible forms of sequences that encode the target protein occur on a separate nucleic acid molecule from the nucleic acid molecules that contain expressible forms of sequences that encode one or more immunomodulatory proteins. In some embodiments, expressible forms of sequences that encode the target protein and expressible forms of sequences that encode one or more of the immunomodulatory proteins  
20       occur on a one nucleic acid molecule that is separate from the nucleic acid molecule that contain expressible forms of sequences that encode one or more of the immunomodulating proteins. Multiple different nucleic acid molecules can be produced and delivered according to the present invention and delivered to the individual. For example, in some embodiments,



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expressible forms of sequences that encode the target protein occur on separate nucleic acid molecule from the nucleic acid molecules that contain expressible forms of sequences that encode one or more of the two immunomodulating proteins which occur on separate nucleic acid molecule from the nucleic acid molecules that contain expressible forms of sequences  
5 that encode one or more immunomodulating proteins. In such cases, all three molecules are delivered to the individual.

The nucleic acid molecule(s) may be provided as plasmid DNA, the nucleic acid molecules of recombinant vectors or as part of the genetic material provided in an attenuated vaccine or cell vaccine. Alternatively, in some embodiments, the target protein and/or wither  
10 or both immunomodulating proteins maybe delivered as a protein in addition to the nucleic acid molecules that encode them or instead of the nucleic acid molecules which encode them.

Genetic constructs may comprise a nucleotide sequence that encodes a target protein or an immunomodulating protein operably linked to regulatory elements needed for gene expression. According to the invention, combinations of gene constructs that include one that  
15 comprises an expressible form of the nucleotide sequence that encodes a target protein and one that includes an expressible form of the nucleotide sequence that encodes an immunomodulating protein are provided. Incorporation into a living cell of the DNA or RNA molecule(s) that include the combination of gene constructs results in the expression of the DNA or RNA and production of the target protein and one or more immunomodulating  
20 proteins. An enhanced immune response against the target protein results.

The present invention may be used to immunize an individual against all pathogens such as viruses, prokaryote and pathogenic eukaryotic organisms such as unicellular pathogenic organisms and multicellular parasites. The present invention is particularly useful

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to immunize an individual against those pathogens which infect cells and which are not encapsulated such as viruses, and prokaryote such as gonorrhea, listeria and shigella. In addition, the present invention is also useful to immunize an individual against protozoan pathogens that include a stage in the life cycle where they are intracellular pathogens. Table 1 provides a listing of some of the viral families and genera for which vaccines according to the present invention can be made. DNA constructs that comprise DNA sequences that encode the peptides that comprise at least an epitope identical or substantially similar to an epitope displayed on a pathogen antigen such as those antigens listed on the tables are useful in vaccines. Moreover, the present invention is also useful to immunize an individual against other pathogens including prokaryotic and eukaryotic protozoan pathogens as well as multicellular parasites such as those listed on Table 2.

In order to produce a genetic vaccine to protect against pathogen infection, genetic material that encodes immunogenic proteins against which a protective immune response can be mounted must be included in a genetic construct as the coding sequence for the target. Whether the pathogen infects intracellularly, for which the present invention is particularly useful, or extracellularly, it is unlikely that all pathogen antigens will elicit a protective response. Because DNA and RNA are both relatively small and can be produced relatively easily, the present invention provides the additional advantage of allowing for vaccination with multiple pathogen antigens. The genetic construct used in the genetic vaccine can include genetic material that encodes many pathogen antigens. For example, several viral genes may be included in a single construct thereby providing multiple targets.

Tables 1 and 2 include lists of some of the pathogenic agents and organisms for which genetic vaccines can be prepared to protect an individual from infection by them. In some

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preferred embodiments, the methods of immunizing an individual against a pathogen are directed against HIV, HSV, HCV, WNV or HBV.

Another aspect of the present invention provides a method of conferring a protective immune response against hyperproliferating cells that are characteristic in hyperproliferative diseases and to a method of treating individuals suffering from hyperproliferative diseases. 5 Examples of hyperproliferative diseases include all forms of cancer and psoriasis.

It has been discovered that introduction of a genetic construct that includes a nucleotide sequence which encodes -an immunogenic "hyperproliferating cell"- associated protein into the cells of an individual results in the production of those proteins in the vaccinated cells of an individual. To immunize against hyperproliferative diseases, a genetic 10 construct that includes a nucleotide sequence that encodes a protein that is associated with a hyperproliferative disease is administered to an individual.

In order for the hyperproliferative-associated protein to be an effective immunogenic target, it must be a protein that is produced exclusively or at higher levels in 15 hyperproliferative cells as compared to normal cells. Target antigens include such proteins, fragments thereof and peptides; which comprise at least an epitope found on such proteins. In some cases, a hyperproliferative-associated protein is the product of a mutation of a gene that encodes a protein. The mutated gene encodes a protein that is nearly identical to the normal protein except it has a slightly different amino acid sequence which results in a different 20 epitope not found on the normal protein. Such target proteins include those which are proteins encoded by oncogenes such as *myb*, *myc*, *fyn*, and the translocation gene *bcr/abl*, *ras*, *src*, P53, *neu*, *trk* and EGRF. In addition to oncogene products as target antigens, target proteins for anti-cancer treatments and protective regimens include variable regions of

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antibodies made by B cell lymphomas and variable regions of T cell receptors of T cell lymphomas which, in some embodiments, are also used target antigens for autoimmune disease. Other tumor-associated proteins can be used as target proteins such as proteins that are found at higher levels in tumor cells including the protein recognized by monoclonal antibody 17-IA and folate binding proteins or PSA.

While the present invention may be used to immunize an individual against one or more of several forms of cancer, the present invention is particularly useful to prophylactically immunize an individual who is predisposed to develop a particular cancer or who has had cancer and is therefore susceptible to a relapse. Developments in genetics and technology as well as epidemiology allow for the determination of probability and risk assessment for the development of cancer in individual. Using genetic screening and/or family health histories, it is possible to predict the probability a particular individual has for developing any one of several types of cancer.

Similarly, those individuals who have already developed cancer and who have been treated to remove the cancer or are otherwise in remission are particularly susceptible to relapse and reoccurrence. As part of a treatment regimen, such individuals can be immunized against the cancer that they have been diagnosed as having had in order to combat a recurrence. Thus, once it is known that an individual has had a type of cancer and is at risk of a relapse, they can be immunized in order to prepare their immune system to combat any future appearance of the cancer.

The present invention provides a method of treating individuals suffering from hyperproliferative diseases. In such methods, the introduction of genetic constructs serves as

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an immunotherapeutic, directing and promoting the immune system of the individual to combat hyperproliferative cells that produce the target protein.

The present invention provides a method of treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response  
5 against targets that are associated with autoimmunity including cell receptors and cells which produce "self"-directed antibodies.

T cell mediated autoimmune diseases include Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma,  
10 polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of the T cells would elicit an immune response including CTLs to eliminate those T cells.

15 In RA, several specific variable regions of T cell receptors (TCRs) that are involved in the disease have been characterized. These TCRs include V $\beta$ -3, V $\beta$ -14, 20 V $\beta$ -17 and Va-17. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in RA. See: Howell, M.D., *et al.*, 1991 *Proc. Nat. Acad. Sci. USA* 88:10921-10925; Piliard, X., *et al.*, 1991 *Science* 253:325-  
20 329; Williams, W.V., *et al.*, 1992 *J Clin. Invest.* 90:326-333; each of which is incorporated herein by reference. In MS, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include V $\beta$ P and Va-10. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response

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that will target T cells involved in MS. See: Wucherpfennig, K.W., *et al.*, 1990 *Science* 248:1016-1019; Oksenberg, J.R., *et al.*, 1990 *Nature* 345:344-346; each of which is incorporated herein by reference.

5 In scleroderma, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include V $\beta$ -6, V $\beta$ -8, V $\beta$ -14 and V $\alpha$ -16, V $\alpha$ -3C, V $\alpha$ -7, V $\alpha$ -14, V $\alpha$ -15, V $\alpha$ -16, V $\alpha$ -28 and V $\alpha$ -12. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in scleroderma.

10 In order to treat patients suffering from a T cell mediated autoimmune disease, particularly those for which the variable region of the TCR has yet to be characterized, a synovial biopsy can be performed. Samples of the T cells present can be taken and the variable region of those TCRs identified using standard techniques. Genetic vaccines can be prepared using this information.

15 B cell mediated autoimmune diseases include Lupus (SLE), Grave's disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulinemia, primary biliary sclerosis and pernicious anemia. Each of these diseases is characterized by antibodies that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of antibodies would elicit an immune response including CTLs to eliminate those B cells that  
20 produce the antibody.

In order to treat patients suffering from a B cell mediated autoimmune disease, the variable region of the antibodies involved in the autoimmune activity must be identified. A biopsy can be performed and samples of the antibodies present at a site of inflammation can

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be taken. The variable region of those antibodies can be identified using standard techniques. Genetic vaccines can be prepared using this information.

In the case of SLE, one antigen is believed to be DNA. Thus, in patients to be immunized against SLE, their sera can be screened for anti-DNA antibodies and a vaccine  
5 can be prepared which includes DNA constructs that encode the variable region of such anti-DNA antibodies found in the sera.

Common structural features among the variable regions of both TCRs and antibodies are well known. The DNA sequence encoding a particular TCR or antibody can generally be found following well known methods such as those described in Kabat, *et al 1987 Sequence*  
10 *of Proteins of Immunological Interest* U.S. Department of Health and Human Services, Bethesda MD, which is incorporated herein by reference. In addition, a general method for cloning functional variable regions from antibodies can be found in Chaudhary, V.K., *et al*, 1990 *Proc. Natl. Acad. Sci. USA* 87:1066, which is incorporated herein by reference.

#### **Recombinant protein production**

15 The present invention relates to in vitro host cell cultures comprising expression vectors operable in such host cells that comprise a nucleic acid sequence that encode fusion proteins that consists of an IgE signal peptide linked to non-IgE protein sequences; to such nucleic acid molecules; and to host cells comprising such vectors. The present invention also relates to methods of producing a fusion proteins comprising the step of culturing the host  
20 cell. The present invention relates to isolated fusion proteins that comprise an IgE signal peptide linked to non-IgE protein sequences.

The fusion proteins may be produced by routine means using readily available starting materials as described above. Provision of a suitable DNA sequence encoding the desired

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protein permits the production of the protein using recombinant techniques now known in the art.

One having ordinary skill in the art can, using well known techniques, insert DNA that encodes a fusion protein into a commercially available expression vector for use in well known expression systems. The commercially available plasmid pYES2 (Invitrogen, San Diego, Calif.) may be used for production in *S. cerevisiae* strains of yeast. The commercially available MaxBac<sup>TM</sup> (Invitrogen, San Diego, Calif.) complete baculovirus expression system may be used for production in insect cells. The commercially available plasmid pcDNA 1 (Invitrogen, San Diego, Calif.) may be used for production in may be used for production in mammalian cells such as Chinese Hamster Ovary cells. One having ordinary skill in the art can use these commercial expression vectors systems or others to produce fusion protein using routine techniques and readily available starting materials.

One having ordinary skill in the art may use other commercially available expression vectors and systems or produce vectors using well known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers, are readily available and known in the art for a variety of hosts. See e.g., Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989). Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

A wide variety of eukaryotic hosts are also now available for production of recombinant foreign proteins. Eukaryotic hosts may be transformed with expression vectors that produce the desired protein directly using the IgE signal peptide.



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Commonly used eukaryotic systems include, but is not limited to, yeast, fungal cells, insect cells, mammalian cells, avian cells, and cells of higher plants. Suitable promoters are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, as e.g. the baculovirus polyhedron promoter. As above,  
5 promoters can be either constitutive or inducible. For example, in mammalian systems, the mouse metallothioneine promoter can be induced by the addition of heavy metal ions.

The particulars for the construction of expression systems suitable for desired hosts are known to those in the art. For recombinant production of the protein, the DNA encoding it is suitably ligated into the expression vector of choice and then used to transform the  
10 compatible host that is then cultured and maintained under conditions wherein expression of the foreign gene takes place. The protein of the present invention thus produced is recovered from the culture, either by lysing the cells or preferably from the culture medium as appropriate and known to those in the art.

One having ordinary skill in the art can, using well known techniques, isolate the  
15 fusion receptor protein or fragments thereof produced using such expression systems.

## EXAMPLES

### Example 1

Human, mouse, and simian IL-15 cDNA encodes a 162 amino acid (aa) residue  
20 precursor protein containing a 48 aa residue leader that is cleaved to generate the 114 aa residue mature IL-15. Human IL-15 shares approximately 97% and 73% sequence identity with simian and mouse IL-15, respectively. Both human and simian IL-15 are active on mouse cells. Although the structure of IL-15 has not been determined, it is predicted to be

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similar to IL-2 and other members of the four-helix bundle cytokine family. (Grabstein, K. et al. (1994) Science 264:965, Anderson, D.M. et al. (1995) Genomics 25:701; and Bamford, R.N. et al. (1995) Cytokine 7:595, Brandhuber, B.J. et al. (1987) Science 238:1707, both of which are incorporated herein by reference.)

5 IL-15 mRNAs have been detected in heart, lung, liver, placenta, skeletal muscle, adherent peripheral blood mononuclear cells, APCs (Dendritic cells), and epithelial and fibroblast cell lines. However, IL-15 mRNA is not detectable in activated peripheral blood T cells that contain high levels of IL-2 mRNA. IL-15 has been shown to stimulate the growth of natural killer cells, activated peripheral blood T lymphocytes, tumor infiltrating lymphocytes (TILs), and B cells. In addition, IL-15 has also been shown to be a chemoattractant for human blood T lymphocytes, to induce lymphokine-activated killer (LAK) activity in NK cells, and to induce the generation of cytolytic effector cells. (Armitage, R.J. et al. (1995) J. Immunol. 154:483; P. Wilkinson and F. Liew (1995) J. Exp. Med. 181:1255; Grabstein, K. et al. (1994) Science 264:965; Giri, J.G. et al. (1994) EMBO J. 13:2822; and Giri, J.G. et al. (1995) EMBO J. 15:3654, each of which is incorporated herein by reference.)

20 Because IL-15 is a prototypic Th1 cytokine, and by virtue of its activity as a stimulator of T cells, NK cells, LAK cells, and TILs, IL-15 is an exciting candidate for use as a molecular adjuvant along with DNA vaccines such as HIV vaccines to enhance cellular immune responses. IL-15 expands HIV specific CTLs, and overproduction of IL-15 is associated with inflammatory diseases such as Crohn's disease.

Northern Blot analysis indicates widespread constitutive expression of IL-15. Control of expression occurs post-transcriptionally at the level of translation and translocation

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(intracellular trafficking). IL-15 mRNA includes a number of elements that impede its translation into protein including: 1) the 5' AUGs are burdened with upstream AUGs that interfere effective IL-15 translation (5 in mice, 12 in humans); 2) the start codon for the IL-15 coding sequence has a weak KOZAK context (GTAATGA); and 3) presence of a negative element in the C-terminus of the IL-15 mature protein coding sequence. (Grabstein et al., (1994) Science 264:965-968, Bamford et al., (1996) PNAS 93:2897-2902; Bamford et al., (1998) J. Immunol 160:4418-4426; and Kozak et al., (1991) J. Cell Biol. 115:887-903, which are each incorporated herein by reference. Each of these three controls may be eliminated to improve expression.

Native IL-15 isoforms contain two leader peptides: a 21 aa signal peptide (SSP) or a 48 aa signal peptide (LSP) (Waldmann et al. Ann. Rev> Immunol. (1999) 17:19-49, which is incorporated herein by reference.

The following strategy for increasing expression of IL-15 through optimization of IL-15 DNA constructs for immunization was followed. Primers were designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message. Primers were designed to include a strong KOZAK context (GCCGCCACC). The C-terminus negative regulatory element was removed using PCR antisense primer design. The primers are set forth in Figure 1.

The following strategy for increasing expression of IL-15 through replacement of the 48 amino acid IL-15 signal peptide (LSP) with IgE leader was performed. Sense primers were designed to start after 48 aa LSP while antisense primer amplified from stop site. Primers were designed to include a strong KOZAK context (GCCGCCACC). Sense primer

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was designed to contain the sequence for IgE leader sequence plus a ATG start site. The primers are set forth in Figure 2.

Various constructs were prepared and used to transfect RD cells. IL-15 protein production was measured for the various constructs. The data is shown in Figure 3, panels A-C. Figure 3, panel A shows a comparison of expression by human constructs that include coding sequences for the 21 amino acid signal peptide linked to IL-15 (IL-15 SSP – left) and coding sequences for the human 48 amino acid signal peptide (IL-15 LSP – right). Figure 3, panel B shows a comparison of expression by human constructs that include coding sequences for the 48 amino acid signal peptide (human IL-15 LSP – left) and coding sequences for the IgE signal peptide (human IL-15-IgE – right). Figure 3, panel C shows a comparison of expression by Macaque constructs that include coding sequences for the 48 amino acid signal peptide (Mac IL-15 LSP – left) and coding sequences for the IgE signal peptide (Mac IL-15-IgE – right).

IL-15 bioactivity was measured of IL-15 protein produced from the various constructs. The data is shown in Figure 4, panels A-B. Figure 4 panel A shows a comparison of IL-15 bioactivity between human constructs that include the 48 amino acid signal peptide (human IL-15 LSP – left) and coding sequences for the IgE signal peptide (human IL-15-IgE – right). Figure 4 panel B shows a comparison of IL-15 bioactivity between Macaque constructs that include coding sequences for the 48 amino acid signal peptide (Mac IL-15 LSP – left) and coding sequences for the IgE signal peptide (Mac IL-15-IgE – right).

A construct was made using the expression vector pVAX with an insertion of an IL-15 coding sequence linked to coding sequence for an IgE signal peptide. Construct encoding HIV-1 Gag were also generated. Immunological experiments were performed comparing the

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effects on immune responses using IL-15 engineered plasmids in combination with HIV-1 Gag. Balb/c Mice were vaccinated according to the immunization schedule shown in Figure 5.

Immune responses were studied by comparing restimulation of antigen-specific IFN- $\gamma$  production 5 weeks following the third immunization. The data is shown in Figure 6. Vaccine groups included naïve mice, mice vaccinated with the vector pCDN3, mice vaccinated with constructs that encoded HIV-1 Gag, mice vaccinated with constructs that encoded HIV-1 Gag and IL-15 linked to the 48 amino acid signal peptide and mice vaccinated with constructs that encoded HIV-1 Gag o linked to the IgE signal peptide.

#### 10 Example 2

An engineered IL-15 plasmid vaccine was constructed by removing the native IL-15 Kozak region, AUG's and UTRs. The engineered IL-15 plasmid was provided with the coding sequence for IgE signal peptide. The engineered IL-15 was expressed at a level 30 to 50 times greater than that observed with a comparable wild type plasmid. The immune response observed in mice co-immunized with engineered IgE signal-IL-15 and HIV-1 gag constructs were significantly times greater than mice immunized with the HIV-1 gag construct alone. Data is shown in Figure 7.

#### Example 3

Isolated cDNA that encodes the immunomodulating proteins are useful as a starting material in the construction of constructs that can produce that immunomodulating protein. In some embodiments, constructs are provided in which coding sequences for one of the following immunomodulatory proteins are linked to the IgE signal peptide. In some

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embodiments, such constructs are provided as part of vaccines and immunomodulating compositions such as those described herein.

Using standard techniques and readily available starting materials, a nucleic acid molecule that encodes an immunomodulating protein may be prepared and incorporated into  
5 constructs, vectors, vaccines etc as described herein.

Genbank Accession number AF031167 refers to the complete coding sequence of human IL-15 mRNA. Genbank Accession numbers Y09908, X91233, X94223 and X94222 also refer to human IL-15 sequences. Each sequence is incorporated herein by reference.

Genbank Accession number L07414 refers to the complete coding sequence of human  
10 CD40-ligand mRNA. The sequence is incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for Bax is L22473, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for TRAIL is U37518 or AF023849 , which are incorporated herein by reference.

15 The GENBANK Accession number for the nucleotide and amino acid sequences for TRAILrecDRC5 is U90875 or AF016266, which are incorporated herein by reference. Also incorporated by reference are TRAIL-R2 AF016849; TRAIL-R3 AF014794; and TRAIL-R4 AF021232.

The GENBANK Accession number for the nucleotide and amino acid sequences for  
20 RANK is AF018253 which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for RANK LIGAND is AF019047 or AF333234, which are incorporated herein by reference.

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The GENBANK Accession number for the nucleotide and amino acid sequences for Ox40 is X75962, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for Ox40 LIGAND is X79929 or AB007839, which are incorporated herein by reference.

5 The GENBANK Accession number for the nucleotide and amino acid sequences for NKG2D is AF461811 or X54870, which are incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for MICA is X92841, which is incorporated herein by reference.

10 The GENBANK Accession number for the nucleotide and amino acid sequences for MICB is U65416, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for NKG2A is X54867, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for NKG2B is X54868, which is incorporated herein by reference.

15 The GENBANK Accession number for the nucleotide and amino acid sequences for NKG2C is X54869 or Aj0016984, which are incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for NKG2E is L14542, which is incorporated herein by reference.

20 The GENBANK Accession number for the nucleotide and amino acid sequences for NKG2F is AH006173, U96845 or U96846, which are incorporated herein by reference.

The GENBANK Accession number for the nucleotide and amino acid sequences for CD30 is M83554, (Durkop, H et al. Cell 68 (3), 421-427 (1992)) which is incorporated herein by reference.

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The GENBANK Accession number for the nucleotide and amino acid sequences for CD153 (CD30L) is L09753, (Smith,C.A., et al. Cell 73 (7), 1349-1360 (1993)) which are incorporated herein by reference

5 The GENBANK Accession number for the nucleotide sequence for Fos is K00650 or V01512, which are each incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for c-jun J04111 or M29039, which are each incorporated herein by reference.

10 The GENBANK Accession number for the nucleotide sequence for Sp-1 is BC021101, BC005250, BC002878, M31126, J02893 or X15102, which are each incorporated herein by reference.

The nucleotide sequence for Ap1 can be identified as described in Lee et al, 1987 Cell 49:741-752, Rauscher et al. 1988 Science 240:1010-1016, and Chiu et al, 1988 Cell 54:541-552, which are each incorporated herein by reference.

15 The GENBANK Accession number for the nucleotide sequence for Ap-2 is M36711, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for p38 is U66243, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for p65Rel is L19067, which is incorporated herein by reference.

20 The GENBANK Accession number for the nucleotide sequence for MyD88 is U70451, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for IRAK is NM001569, which is incorporated herein by reference.



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The GENBANK Accession number for the nucleotide sequence for TRAF6 is U78798, which is incorporated herein by reference.

The nucleotide sequence for Ikb can be found as described in Gilmore et al. Trends Genet 1993 Dec;9(12):427-33, which is incorporated herein by reference.

5 The GENBANK Accession number for the nucleotide sequence for NIK is Y10256, which is incorporated herein by reference.

The nucleotide sequence for SAP K can be found as described in Franklin et al. Oncogene. 1995 Dec 7;11(11):2365-74. which is incorporated herein by reference.

10 The GENBANK Accession number for the nucleotide sequence for SAP1 is M85164 or M85165, which are each incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for JNK2 is L31951, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for JNK1B2 is U35005; which is incorporated herein by reference.

15 The GENBANK Accession number for the nucleotide sequence for JNK1B1 is U35004; which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for JNK2B2 is U35003; which is incorporated herein by reference.

20 The GENBANK Accession number for the nucleotide sequence for JNK2B1 is U35002; which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for JNK1A2 is U34822; which is incorporated herein by reference.

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The GENBANK Accession number for the nucleotide sequence for JNK2A1 is U34821; which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for JNK3A1 is U34820; which is incorporated herein by reference.

5 The GENBANK Accession number for the nucleotide sequence for JNK3A2 is U34819, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for NF-kappa-B2, p49 splice form is A57034, which is incorporated herein by reference.

10 The GENBANK Accession number for the nucleotide sequence for NF-kappa-B2, p100 splice form is A42024, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for NF-kappa-B2, p105 splice form is S17233, which is incorporated herein by reference.

The GENBANK Accession number for the nucleotide sequence for NF-kappa-B 50K chain precursor is A37867, which is incorporated herein by reference.

15 The nucleotide sequence for NFkB p50 is described in Meyer R., et al. (1991) Proc. Natl. Acad. Sci. USA 88(3), 966 970, which is incorporated herein by reference.

The nucleotide and amino acid sequences of human IL-1  $\alpha$  are well known and set forth in Telford, et al. (1986) Nucl. Acids Res. 14:9955-9963, Furutani, et al. (1985) Nucl. Acids Res. 14:3167-3179, March, et al. (1985) Nature 315:641-647, and accession code  
20 Swissprot PO1583, which are each incorporated herein by reference.

The nucleotide and amino acid sequences of human IL-2 are well known and set forth in Holbrook, et al. (1984) Proc. Natl. Acad. Sci. USA 81:1634-1638, Fujita, et al. (1983) Proc. Natl. Acad. Sci. USA 80:7437-7441, Fuse, et al. (1984) Nucl. Acids Res. 12:9323-

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9331, Taniguchi, et al. (1983) nature 302:305-310, Meada, et al. (1983) Biochem. Biophys. Res. Comm. 115:1040-1047, Devos, et al. (1983) Nucl. Acids Res. 11:4307-4323, and accession code Swissprot PO1585, which are each incorporated herein by reference.

The nucleotide and amino acid sequences of human IL-4 are well known and set forth in Arai, et al. (1989) J. Immunol. 142:274-282 Otsuka, et al. (1987) Nucl. Acids Res. 15:333-344, Yokota, et al. (1986) Proc. Natl. Acad. Sci USA 83:5894-5898, Noma, et al. (1984) Nature 319:640-646, Lee, et al. (1986) Proc. Natl. Acad. Sci. USA 83:2061-2063, and accession code Swissprot 05112 (the accession code for murine IL-4 is Swissprot 07750), Which are each incorporated herein by reference.

10 The nucleotide and amino acid sequences of human IL-5 are well known and set forth in Campbell, et al. (1987) Proc. Natl. Acad. Sci. USA 84:6629-6633, Tanabe, et al. (1987) J. Biol. Chem. 262:16580-16584, Campbell, et al. (1988) Eur. J. Biochem. 174:345-352, Azuma, et al. (1986) Nucl. Acids Res. 14:9149-9158, Yokota, et al. (1986) Proc. Natl. Acad. Sci. USA 84:7388-7392, and accession code Swissprot PO5113, which are each incorporated  
15 herein by reference.

The nucleotide and amino acid sequences of human IL-10 are well known and set forth in Viera, et al. (1991) Proc. Natl. Acad. Sci. USA 88:1172-1176, and accession code Swissprot P22301.

The nucleotide and amino acid sequences of human IL-15 are well known and set  
20 forth in Grabstein, et al. (1994) Science 264:965-968, and accession code Swissprot UO3099, which are each incorporated herein by reference.

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The nucleotide and amino acid sequences of human IL-18 are well known and set forth in Ushio, et al. (1996) J. Immunol. 156:4274-4279, and accession code D49950, which are each incorporated herein by reference.

5 The nucleotide and amino acid sequences of human TNF-  $\alpha$  are well known and set forth in Pennica, (1984) Nature 312:724-729, and accession code Swissprot PO1375, which are each incorporated herein by reference.

The nucleotide and amino acid sequences of human TNF-  $\beta$  are well known and set forth in Gray, (1984) Nature 312:721-724, and accession code Swessprot PO1374, which are each incorporated herein by referenccc. ino acid sequences of human IL-10 are well know and  
10 set forth in Viera, et al. (1991) Proc. Natl. Acad. Sci. USA 88:1172-1176, and accession code Swissprot P22301, which are each incorporated herein by reference.

The complete coding sequence for human interleukin 12 mRNA, is set forth in Genbank Accession Number AF180563 (P40 mRNA) and AF180562 (P35 mRNA) and U.S. Patent Number 5,840,530, which are each incorporated herein by reference.

15 Sequence information for MadCAM-1 is found at Genbank Accession Number U80016 (Leung, E., et al, Immunogenetics 46 (2), 111-119 (1997)) , which are each incorporated herein by reference.

Sequence information for MadCAM-1 is found at Genbank Accession Number U43628 (Shyjan, A.M., et al, J. Immunol. 156 (8), 2851-2857 (1996)) , which are each  
20 incorporated herein by reference.

Sequence information for NGF is found at Genbank Accession Number M57399 (Kretschmer, P.J., et al., Growth Factors 5, 99-114 (1991)) , which are each incorporated herein by reference.

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Sequence information for IL-7 is found at Genbank Accession Number J04156 (Goodwin, R.G., et al., Proc. Natl. Acad. Sci. U.S.A. 86 (1), 302-306 (1989)) , which are each incorporated herein by reference.

5 Sequence information for VEGF is found at Genbank Accession Number M32977 (Leung, D.W., et al., Science 246, 1306-1309 (1989)) , which are each incorporated herein by reference.

Sequence information for TNF-R is found at Genbank Accession Number M60275 (Gray, P.W., et al. Proc. Natl. Acad. Sci. U.S.A. 87, 7380-7384 (1990)) , which are each incorporated herein by reference.

10 Sequence information for TNF-R is found at Genbank Accession Number M63121 (Himmeler, A., et al. DNA Cell Biol. 9, 705-715 (1990)), which are each incorporated herein by reference.

Sequence information for Fas is found at Genbank Accession Number M67454 (Itoh, N., et al., Cell 66 (2), 233-243 (1991)), which are each incorporated herein by reference.

15 Sequence information for CD40L is found at Genbank Accession Number L07414 (Gauchat, J.F.M., et al. FEBS Lett, 315, 259-266 (1992)), which are each incorporated herein by reference.

20 Sequence information for IL-4 is found at Genbank Accession Number M23442 (Arai, N., et al., J. Immunol. 142 (1), 274-282 (1989)), which are each incorporated herein by reference.

Sequence information for IL-4 is found at Genbank Accession Number M13982 (Yokota, T., et al. Proc. Natl. Acad. Sci. U.S.A. 83 (16), 5894-5898 (1986)), which are each incorporated herein by reference.

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Sequence information for CSF is found at Genbank Accession Number M37435 (Wong, G.G., et al. Science 235 (4795), 1504-1508 (1987)), which are each incorporated herein by reference.

Sequence information for G-CSF is found at Genbank Accession Number X03656  
5 (Nagata, S., et al, EMBO J. 5 (3), 575-581 (1986)), which are each incorporated herein by reference.

Sequence information for G-CSF is found at Genbank Accession Number X03655 (Nagata, S., et al., EMBO J. 5 (3), 575-581 (1986)), which are incorporated herein by reference.

10 Sequence information for GM-CSF is found at Genbank Accession Number M11220 (Lee, F., et al., Proc. Ntl. Acad. Sci. U.S.A. (13), 4360-4364 (1985)) which are incorporated herein by reference.

Sequence information for GM-CSF is found at Genbank Accession Number M10663 (Wong, G.G., et al., Science 228 (4701), 810-815 (1985)) which are incorporated herein by  
15 reference.

Sequence information for M-CSF is found at Genbank Accession Number M27087 (Takahashi, M., et al., Biochem. Biophys. Res. Commun. 161 (2), 892-901 (1989)) which are incorporated herein by reference.

Sequence information for M-CSF is found at Genbank Accession Number M37435  
20 (Wong G.G., et al., Science 235 (4795), 1504-1508 (1987)) which are incorporated herein by reference.

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Sequence information for LFA-3 is found at Genbank Accession Number Y00636 (Wallner, B.P., et al., J. Exp. Med. 166 (4), 923-932 (1987)) which are incorporated herein by reference.

Sequence information for ICAM-3 is found at Genbank Accession Number X69819  
5 which are incorporated herein by reference.

Sequence information for ICAM-2 is found at Genbank Accession Number X15606 (Staunton, D.E., et al., Nature 339 (6219), 61-64 (1989)) which are incorporated herein by reference.

Sequence information for ICAM-1 is found at Genbank Accession Number J03132  
10 (Staunton, D.E., et al., Cell 52 (6), 925-933 (1988)) which are incorporated herein by reference.

Sequence information for PECAM is found at Genbank Accession Number M28526 (Newman, P.J., et al., Science 247, 1219-1222 (1990)) which are incorporated herein by reference.

15 Sequence information for P150.95 is found at Genbank Accession Number Y00093 (Corbi, A.L., et al., EMBO J. 6 (13), 4023-4028 (1987)) which are incorporated herein by reference.

Sequence information for Mac-1 is found at Genbank Accession Number J03925 (Corbi, A.L., et al., J. Biol. Chem. 263 (25), 12403-12411 (1988)) which are incorporated  
20 herein by reference.

Sequence information for LFA-1 is found at Genbank Accession Number Y00796 (Larson, R., et al., J. Cell Biol. 108 (2), 703-712 (1989)) which are incorporated herein by reference.

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Sequence information for CD34 is found at Genbank Accession Number M81104 (Simmons, D.L. et al., J. Immunol. 148, 267-271 (1992)) which are incorporated herein by reference.

Sequence information for RANTES is found at Genbank Accession Number M21121  
5 (Schall, T.J., et al., J. Immunol. 141, 1018-1025 (1988)) which are incorporated herein by reference.

Sequence information for IL-8 is found at Genbank Accession Number M28130 (Mukaida, N., et al., J. Immunol. 143 (4), 1366-1371 (1989)) which are incorporated herein by reference.

10 Sequence information for MIP-1 $\alpha$  is found at Genbank Accession Number U72395 (Fridell, R.A., et al., J. Cell. Sci 110 (pt 11), 1325-1331 (1997)) which are incorporated herein by reference.

Sequence information for E-selectin is found at Genbank Accession Number M24736 (Bevilacqua, M.P., et al., Science 243 (4895), 1160-1165 (1989)) which are incorporated  
15 herein by reference.

Sequence information for CD2 is found at Genbank Accession Number M14362 (Sewell, W.A., et al. Proc. Natl. Acad. Sci. U.S.A. 83, 8718-8722 (1986); Proc. Natl. Acad. Sci. U.S.A. 84, 7256-7256 (1987)) which are incorporated herein by reference.

Sequence information for MCP-1 is found at Genbank Accession Number S69738  
20 (Li, Y.S., et al., Mol. Cell. Biochem. 126 (1), 61-68 (1993)) which are incorporated herein by reference.



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Sequence information for L-selection is found at Genbank Accession Number X16150 (Tedder, T.F., et al., J. Exp. Med. 170 (1), 123-133 (1989)) which are incorporated herein by reference.

Sequence information for P-selection is found at Genbank Accession Number  
5 M25322 (Johnston, G.I., et al., Cell 56, 1033-1044 (1989)) which are incorporated herein by reference.

Sequence information for FLT is found at Genbank Accession Number X94263 (Mandriota, S.J., et al., J. Biol. Chem. 271 (19), 11500-11505 (1996)) which are incorporated herein by reference.

10 Sequence information for FLT is found at Genbank Accession Number X51602 (Shibuya, M. et al. Oncogene 5 (4), 519-524 (1990) Han, H.J., et al. Hum. Mol. Genet. 2 (12), 2204 (1993)) which are incorporated herein by reference.

Sequence information for Apo-1 is found at Genbank Accession Number X63717 (Oehm, et al, J. Biol.Chem., (1992), 267 (15), 10709-15) which are incorporated herein by  
15 reference.

Sequence information for Fas is found at Genbank Accession Number M67454 (Itoh, et al., Cell, (1991), 66 (2), 233-43) which are incorporated herein by reference.

Sequence information for TNFR-1 is found at Genbank Accession Number M67454 (Nophar, et al., EMBO J., 1990, 9(10), 3269-78) which are incorporated herein by reference.

20 Sequence information for p55 is found at Genbank Accession Number M58286 (Loetscher, et al., Cell, 1990, 61, 351-359) which are incorporated herein by reference.

Sequence information for WSL-1, is found at Genbank Accession Number Y09392 (Kitson, et al., Nature, 1996, 384 (6607), 372-5) which are incorporated herein by reference.

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Sequence information for DR3 is found at Genbank Accession Number U72763 (Chinnaiyan, et al., Science, 1996, 274 (5829), 990-2) which are incorporated herein by reference.

Sequence information for TRAMP is found at Genbank Accession Number U75381  
5 (Bodmer, et al., Immunity, 1997, 6 (1), 79-88) which are incorporated herein by reference.

Sequence information for Apo-3 is found at Genbank Accession Number U74611 (Marsters, et al., Curr. Biol., 1996, 6 (12), 1669-76) which are incorporated herein by reference.

Sequence information for AIR is found at Genbank Accession Number U78029 which  
10 is incorporated herein by reference.

Sequence information for LARD is found at Genbank Accession Number U94512 (Screaton, et al., Proc. Natl. Acad. Sci. USA, 1997, 94(9), 4615-19) which are incorporated herein by reference.

Sequence information for NGRF is found at Genbank Accession Number M14764  
15 (Johnson, et al., Cell, 1986, 47(4), 545-554) which are incorporated herein by reference.

Sequence information for DR4(TRAIL) is found at Genbank Accession Number U90875 (Pan, et al., Science, 1997, 276(5309), 111-113) which are incorporated herein by reference.

Sequence information for DR5 is found at Genbank Accession Number AF012535  
20 (Sheridan, et al. , Science, 1997, 277(5327), 818-821) which are incorporated herein by reference.

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Sequence information for KILLER is found at Genbank Accession Number AF022386 (Wu, et al., Nat. Genet. 17 (2), 141-143 (1997)) which are incorporated herein by reference.

Sequence information for TRAIL-R2 is found at Genbank Accession Number  
5 AF020501 which is incorporated herein by reference.

Sequence information for TRICK2 is found at Genbank Accession Number AF018657.

Sequence information for DR6 is found at Genbank Accession Number AF068868 which is incorporated herein by reference.

10 Sequence information for ICE is found at Genbank Accession Numbers U13697, U13698 and U13699 (Alnemri, E.S., et al., J. Biol. Chem. 270 (9), 4312-4317 (1995)) which are incorporated herein by reference.

Sequence information for VLA-1 is found at Genbank Accession Number X17033 (Takada., et al., J. Biol. Chem. 109 (1), 397-407 (1989)) which are incorporated herein by  
15 reference.

Sequence information for CD86 (B7.2) is found at Genbank Accession Number U04343 (Azuma, et al., Nature. 366 (6450), 76 (1993)) which are incorporated herein by reference.

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Table 1

Picornavirus Family	
cold.	<p>Genera: Rhinoviruses: (Medical) responsible for - 50% cases of the common</p> <p>5 Etheroviruses: (Medical) includes polioviruses, coxsackieviruses, cchoviruses, and human enteroviruses such as hepatitis A virus.</p> <p>Apthoviruses: (Veterinary) these are the foot and mouth disease viruses.</p> <p>Target antigens: VP1, VP2, VP3, VP4, VPG</p>
10 Calcivirus Family	
	<p>Genera: Norwalk Group of Viruses: (Medical) these viruses are an important causative agent of epidemic gastroenteritis.</p>
Togavirus Family	
15	<p>Genera: Alphaviruses: (Medical and Veterinary) examples include Senilis viruses, RossRiver virus and Eastern &amp; Western Equine encephalitis.</p> <p>Reovirug: (Medical) Rubella virus.</p>
Flariviridue Family	
20	<p>Examples include: (Medical) dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses. West Nile virus (Genbank NC001563, AF533540, AF404757, AF404756, AF404755, AF404754, AF404753, AF481864, M12294, AF317203, AF196835, AF260969, AF260968, AF260967, AF206518 and AF202541)</p>
25	<p>Representative Target antigens:</p> <p>E</p> <p>NS5</p> <p>C</p>
30	<p>Hepatitis C Virus: (Medical) these viruses are not placed in a family yet but are believed to be either a togavirus or a flavivirus. Most similarity is with togavirus family.</p>
Coronavirus Family: (Medical and Veterinary)	
35	<p>Infectious bronchitis virus (poultry)</p> <p>Porcine transmissible gastroenteric virus (pig)</p> <p>Porcine hemagglutinating encephalomyelitis virus (pig)</p> <p>Feline infectious peritonitis virus (cats)</p>

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Feline enteric coronavirus (cat)  
 Canine coronavirus (dog)  
 SARS associated coronavirus  
 The human respiratory coronaviruses cause ~40 cases of common cold.  
 EX. 224E, OC43  
 Note - coronaviruses may cause non-A, B or C hepatitis

## Target antigens:

E1 - also called M or matrix protein  
 E2 - also called S or Spike protein  
 E3 - also called BE or hemagglutinin-esterase  
 glycoprotein (not present in all coronaviruses)  
 N - nucleocapsid

## Rhabdovirus Family

Genera: Vesiliovirus  
 Lyssavirus: (medical and veterinary)  
 rabies

Target antigen: G protein  
 N protein

## Filoviridae Family: (Medical)

Hemorrhagic fever viruses such as Marburg and Ebola virus

## Paramyxovirus Family:

Genera: Paramyxovirus: (Medical and Veterinary)  
 Mumps virus, New Castle disease virus (important pathogen in chickens)  
 Morbillivirus: (Medical and Veterinary)  
 Measles, canine distemper  
 Pneumovirus: (Medical and Veterinary)  
 Respiratory syncytial virus

## Orthomyxovirus Family (Medical)

The Influenza virus

## Bunyavirus Family

Genera: Bunyavirus: (Medical) California encephalitis, LA

Crosse

Phlebovirus: (Medical) Rift Valley Fever  
 Hantavirus: Puumala is a hemorrhagic fever virus  
 Nairovirus (Veterinary) Nairobi sheep disease  
 Also many unassigned bunyaviruses

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## Arnavirus Family (Medical)

LCM, Lassi fever virus

## Reovirus Family

## Genera:

Reovirus: a possible human pathogen  
 Rotavirus: acute gastroenteritis in children  
 Orbiviruses: (Medical and Veterinary)  
 Colorado Tick fever, Lebombo (humans) equine  
 encephalosis, blue tongue

## Retrovirus Family

## Sub-Family:

Oncorivirinal: (Veterinary) (Medical) feline leukemia  
 virus, HTLV I and HTLV II  
 Lentivirinal: (Medical and Veterinary) HIV, feline  
 immunodeficiency virus, equine infections, anemia

Spumavirinal

## Papovavirus Family

## Sub-Family:

Polyomaviruses: (Medical) BKU and JCU viruses

## Sub-Family:

Papillomavirus: (Medical) many viral types associated  
with cancers or malignant progression of papilloma.

## Adenovirus (Medical)

EX AD7, ARD., O.B. - cause respiratory disease - some  
 adenoviruses such as 275 cause enteritis

## Parvovirus Family (Veterinary)

Feline parvovirus: causes feline enteritis

Feline panleucopeniavirus

Canine parvovirus

Porcine parvovirus

## Herpesvirus Family

Sub-Family: alphaherpesviridae

Genera: Simplexvirus (Medical)

HSV I (Genbank X14112, NC001806), HSV II

Varicellovirinis: (Medical Veterinary) pseudorabies -  
 varicella zoster

Sub-Family - betaherpesviridae

Genera: Cytomegalovirus (Medical)

HCMV

Muromegalovirus

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	Sub-Family:	Gammaherpesviridae
	Genera:	Lymphocryptovirus (Medical)
		EBV - (Burkitts lympho)
		Rhadinovirus
5	Poxvirus Family	
	Sub-Family:	Chordopoxviridae (Medical - Veterinary)
	Genera:	Variola (Smallpox)
		Vaccinia (Cowpox)
		Parapoxvirus - Veterinary
10		Auipoxvirus - Veterinary
		Capripoxvirus
		Leporipoxvirus
		Suipoxvirus
	Sub-Family:	Entomopoxviridae
15	Hepadnavirus Family	
		Hepatitis B virus
	Unclassified	Hepatitis delta virus

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Table 2

## Bacterial pathogens

5	Pathogenic gram-positive cocci include: pneumococcal; staphylococcal; and streptococcal. Pathogenic gram-negative cocci include: meningococcal; and gonococcal.
10	Pathogenic enteric gram-negative bacilli include: enterobacteriaceae; pseudomonas, acinetobacteria and eikenella, melioidosis, salmonella; shigellosis; hemophilus; chancroid; brucellosis; tularemia; yersinia (pasteurella); streptobacillus mortiliformis and spirillum; listeria monocytogenes; crysiopelothrix rhusiopathiae; diphtheria, cholera, anthrax; donovanosis (granuloma inguinale); and bartonellosis.
15	Pathogenic anaerobic bacteria include: tetanus; botulism; other clostridia; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include: syphilis; - treponematoses: yaws, pinta and endemic syphilis; and leptospirosis.
20	Other infections caused by higher pathogen bacteria and pathogenic fungi include: actinomycosis; nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidioidomycosis, petriellidiosis, torulopsosis, mycetoma, and chromomycosis; and dermatophytosis.
25	Rickettsial infections include rickettsial and rickettsioses.
30	Examples of mycoplasma and chlamydial infections include: mycoplasma pneumoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections.
	Pathogenic eukaryotes
35	Pathogenic protozoans and helminths and infections thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; pneumocystis carinii; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections.



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**Claims**

1. An isolated nucleic acid molecule comprising a nucleic acid sequence that encodes a fusion protein that consists of an IgE signal peptide linked to non-IgE protein sequences where in the IgE signal peptide and the non-IgE protein sequences are derived from the same  
5 species of animal.
2. An isolated nucleic acid molecule comprising a nucleic acid sequence that encodes a fusion protein that consists of an IgE signal peptide linked to non-IgE protein sequences wherein the non-IgE protein is an enzyme or functional fragment thereof.
- 10 3. An isolated nucleic acid molecule comprising a nucleic acid sequence that encodes a fusion protein that consists of an IgE signal peptide linked to non-IgE protein sequences wherein the non-IgE protein is an immunomodulating protein or functional fragment thereof.
- 15 4. The isolated nucleic acid molecule of claim 3 wherein the fusion protein consists of an IgE signal peptide linked to an immunomodulating protein or functional fragment thereof.
5. The isolated nucleic acid molecule of claims 3-4 wherein the IgE signal peptide and the non-IgE protein sequences are derived from the same species of animal.
- 20 6. The isolated nucleic acid molecule of claims 3-5 wherein the immunomodulating protein or functional fragment thereof is IL-15 protein or functional fragment thereof.

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7. The isolated nucleic acid molecule of claim 6 wherein the is IL-15 protein or functional fragment thereof is IL-15 protein free of IL-15 signal peptide or a functional fragment of IL-15 protein free of IL-15 signal peptide.
- 5 8. The isolated nucleic acid molecule of claims 6-7 wherein the nucleic acid sequence that encodes the IL-15 protein or functional fragment thereof is free of IL-15 Kozak region and/or IL-15 5' untranslated region and/or IL-15 3' untranslated region.
- 10 9. The isolated nucleic acid molecule of claims 6-8 further comprising a nucleotide sequence that encodes CD40L or a functional fragment thereof.
10. The isolated nucleic acid molecule of claims 3-8 further comprising a nucleic acid sequence that encodes an immunogen.
- 15 11. The isolated nucleic acid molecule of claim 10 wherein said immunogen is a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases.
- 20 12. The isolated nucleic acid molecule of claim 11 wherein said immunogen is a pathogen antigen.
13. The isolated nucleic acid molecule of claim 12 wherein said immunogen is a pathogen antigen from a pathogen selected from the group consisting of HIV, HSV, HCV, and WNV.

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14. The isolated nucleic acid molecule of claims 1-13 wherein said isolated nucleic acid molecule is a plasmid.
- 5 15. The nucleic acid molecule of claims 1-14 incorporated into a viral vector.
16. A composition comprising a nucleic acid molecule of claims 3-9, and a nucleic acid molecule that comprises a nucleic acid sequence that encodes an immunogen.
- 10 17. The composition of claim 16 wherein said immunogen is a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases.
18. The composition of claim 17 wherein said immunogen is a pathogen antigen.
- 15 19. The composition of claim 18 wherein said immunogen is a pathogen antigen from a pathogen selected from the group consisting of HIV, HSV, HCV, and WNV.
20. The composition of claims 16-19 wherein a nucleic acid molecule further comprises a nucleotide sequence that encodes CD40L.
- 20 21. The composition of claims 16-19 further comprising a nucleic acid molecule that comprises a nucleotide sequence that encodes CD40L.

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22. A composition comprising a nucleic acid molecule of claim 3-10 further comprising a nucleic acid molecule that comprises a nucleotide sequence that encodes CD40L.

23. The composition of claims 16-22 wherein said nucleic acid molecules are plasmids.

5

24. An injectable pharmaceutical composition comprising the nucleic acid molecules of claims 1-15 or the composition of claims 16-22.

10 25. A recombinant vaccine comprising a nucleic acid sequence that encodes an immunogen and a nucleic acid sequence that encodes a fusion protein comprising an IgE signal sequence linked to an immunomodulating protein or a functional fragment thereof.

26. The recombinant vaccine of claim 25 wherein the fusion protein consists of an IgE signal sequence linked to an immunomodulating protein or a functional fragment thereof.

15

27. The recombinant vaccine of claims 25-26 wherein the immunomodulating protein or a functional fragment thereof is IL-15 protein or a functional fragment thereof.

20 28. The recombinant vaccine of claim 27 wherein IL-15 protein or a functional fragment thereof is IL-15 protein free of IL-15 signal peptide or a functional fragment of IL-15 protein free of IL-15 signal peptide.

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29. The recombinant vaccine of claims 27-28 wherein the nucleic acid sequence that encodes the IL-15 protein or functional fragment thereof is free of IL-15 Kozak region and/or IL-15 5' untranslated region and/or IL-15 3' untranslated region.

5 30. The recombinant vaccine of claims 27-29 further comprising a nucleotide sequence that encodes CD40L.

31. The recombinant vaccine of claims 25-30 wherein said immunogen is a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune  
10 diseases.

32. The recombinant vaccine of claim 31 wherein said immunogen is a pathogen antigen.

33. The recombinant vaccine of claim 31 wherein said pathogen antigen from a pathogen  
15 selected from the group consisting of HIV, HSV, HCV, and WNV.

34. The recombinant vaccine of claims 25-33 wherein said recombinant vaccine is a recombinant vaccinia vaccine.

20 35. A method of inducing an immune response in an individual against an immunogen comprising administering to said individual a nucleic acid molecule of claims 10-15, or a composition of claims 16-24 or a recombinant vaccine of claims 25-34.

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36. A live attenuated pathogen comprising a nucleotide sequence that encodes a fusion protein comprising an IgE signal peptide linked to an immunomodulating protein or a functional fragment thereof.

5 37. The live attenuated pathogen of claim 36 wherein the fusion protein consists of an IgE signal peptide linked to an immunomodulating protein or a functional fragment thereof.

38. The live attenuated pathogen of claims 36-37 wherein the immunomodulating protein or a functional fragment thereof is IL-15 protein or a functional fragment thereof.

10 39. The live attenuated pathogen of claim 38 wherein IL-15 protein or a functional fragment thereof is IL-15 protein free of IL-15 signal peptide or a functional fragment of IL-15 protein free of IL-15 signal peptide.

15 40. The live attenuated pathogen of claims 38-39 wherein the nucleic acid sequence that encodes the IL-15 protein or functional fragment thereof is free of IL-15 Kozak region and/or IL-15 5' untranslated region and/or IL-15 3' untranslated region.

20 41. The live attenuated pathogen of claims 38-40 further comprising a nucleotide sequence that encodes CD40L.

42. A method of immunizing an individual against a pathogen comprising administering to said individual the live attenuated pathogen of claims 36-41.

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43. A method of modulating an immune response in an individual comprising administering to said individual a nucleic acid molecule of claims 3-9 or a composition of claim 22.

5 44. A fusion protein comprising an IgE signal peptide linked to non-IgE protein wherein the IgE signal peptide and the non-IgE protein are derived from the same species of animal.

45. A fusion protein comprising an IgE signal peptide linked to non-IgE protein wherein the non-IgE protein is an enzyme or a functional fragment thereof.

10

46. The fusion protein of claim 44 wherein the non-IgE protein sequence is an immunomodulating protein or a functional fragment thereof.

15 47. The fusion protein of claim 44 consisting of an IgE signal peptide linked to an immunomodulating protein or a functional fragment thereof

48. The fusion protein of claims 46-47 wherein the immunomodulating protein or a functional fragment thereof is IL-15 protein or a functional fragment thereof.

20 49. The fusion protein of claim 48 wherein IL-15 protein or a functional fragment thereof is IL-15 protein free of IL-15 signal peptide or a functional fragment of IL-15 protein free of IL-15 signal peptide.

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50. The fusion protein of claims 45-49 wherein the IgE signal peptide and the non-IgE protein are derived from the same species of animal.

5 51. An in vitro cell culture that comprises cells that comprise a nucleic acid molecule comprising a nucleic acid sequence that encodes a fusion protein that consists of an IgE signal peptide linked to non-IgE protein sequences wherein the nucleic acid sequence is operably linked to regulatory elements required for expression in said cells.

10 52. A method of preparing a non-IgE protein comprising culturing cells that comprise cells that comprises cells that comprise a nucleic acid molecule comprising a nucleic acid sequence that encodes a fusion protein that consists of an IgE signal peptide linked to non-IgE protein sequences wherein the nucleic acid sequence is operably linked to regulatory elements required for expression in said cells under condition necessary for fusion protein expression for a period sufficient for said cells to express said fusion protein.

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**ABSTRACT**

Fusion proteins and nucleic acid molecules encoding fusion proteins are disclosed. The fusion proteins comprise an IgE signal peptide linked to non-IgE protein sequences. Vectors comprising such nucleic acid molecules; and to host cells comprising such vectors  
5 are disclosed as well as methods of using such nucleic acid molecules alone or in combination with nucleic acid sequences encoding immunogens, which are part of the nucleic acid molecules and/or part of a different nucleic acid molecule. Recombinant vaccines and live attenuated pathogens encoding fusion proteins, and methods of using the same, are disclosed.

# Strategy for Increasing Expression of IL-15 through Optimization of IL-15 DNA Constructs for Immunization

- ❖ Primers are designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Removal of the C-terminus negative regulatory element using PCR antisense primer design

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15 (LSP)	sense	GCCCCCGTCGACGCCGCCACCATGAGAAATTCGAAACCAATTGAG
	antisense	ATCGGGCTCGAGTCAAGAAAGTGTGATGAACATTTGG
Macaque IL-15 (LSP)	sense	GCCCCCGTCGACGCCGCCACCATGAGAAATTCGAAACCAATTGAG
	antisense	ATCGGGCTCGAGTCAAGAAAGTGTGATGAACATTTGG
Human IL-15 (SSP)	sense	GCCCCCGGTACC GCCGCCACCATGGTATTGGGAACCATA
	antisense	ATCGGGGGATCC TCAAGAAAGTGTGATGAACAT
Legend: Restriction Site, KOZAK, START, STOP CODON		

Figure 1

# Strategy for Increasing Expression of IL-15 through Replacement of 48 amino acid Signal Peptide (LSP) with IgE leader

- ❖ Sense primers are designed to start after 48 aa ISP while antisense primer amplifies from stop site.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Sense primer is designed to contain the sequence for IgE leader sequence plus a ATG start site.

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15-IgE	sense	GCCCCCGAATTC GCCGCCACCATTGGACTTGGATCTTATTTT
		AGTTGCTGCTGCTACTAGAGTTCATTCTAACTGGTGAATGTAATAAGT
	antisense	ATCGGGCTCGAG TCAAGAAGTGTTGATGAACATTTGG
Macaque IL-15-IgE	sense	GCCCCCGAATTC GCCGCCACCATTGGACTTGGATCTTATTTT
		AGTTGCTGCTGCTACTAGAGTTCATTCTAACTGGTGAATGTAATAAGT
	antisense	ATCGGGCTCGAG TCAAGAAGTGTTGATGAACATTTGG

Legend: Restriction Site, KOZAK, START, STOP CODON

Figure 2

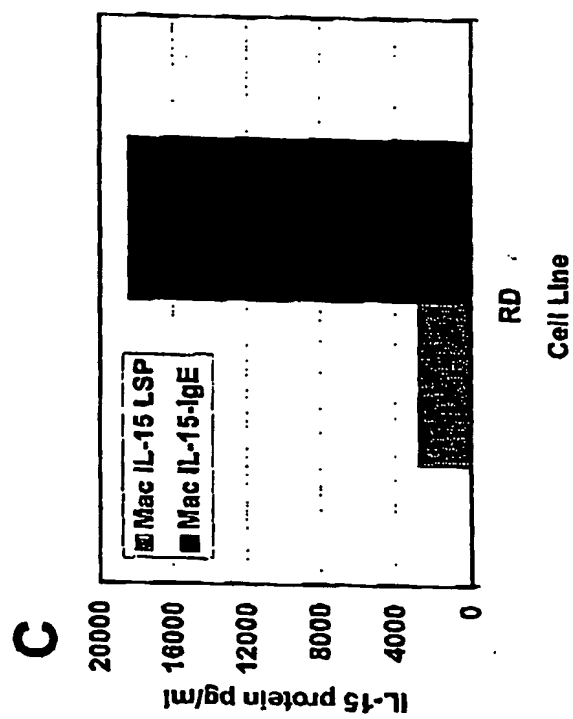
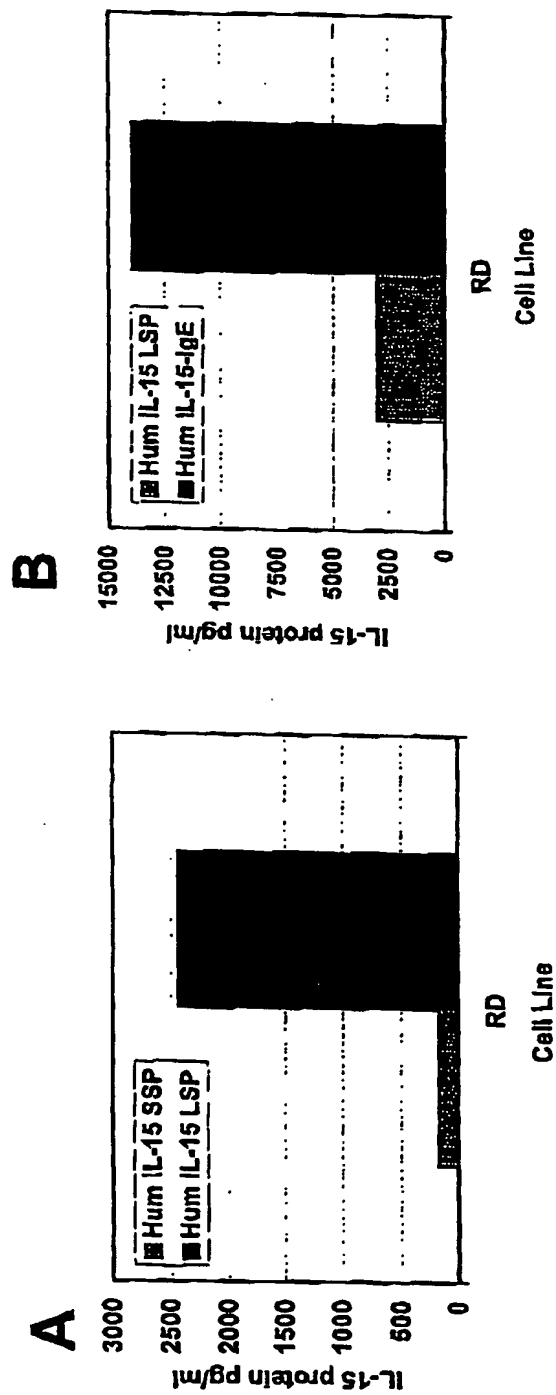


Figure 3

Figure 4

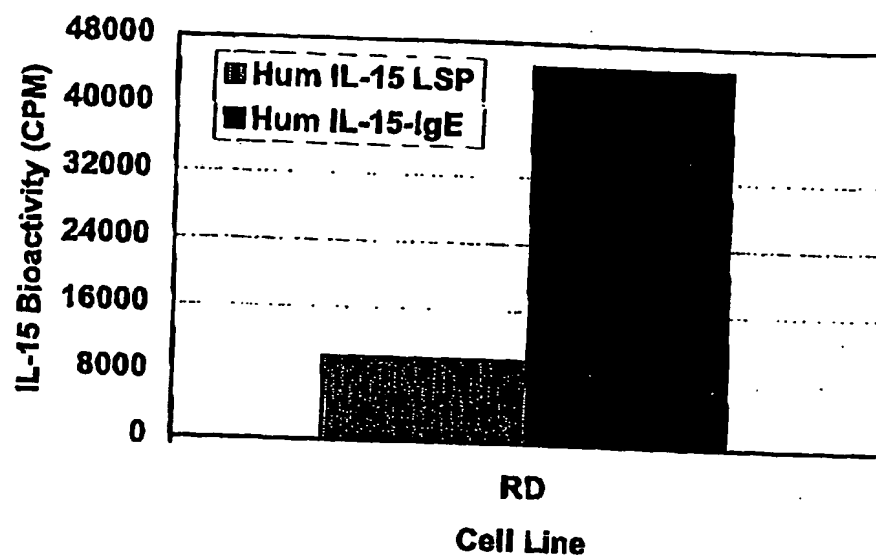
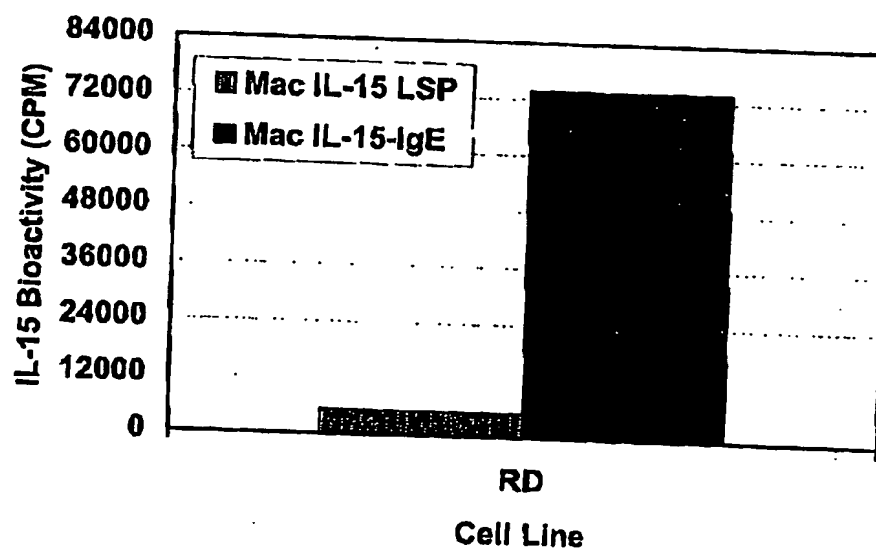
**A****B**

Figure 5

# Immunization Schedule

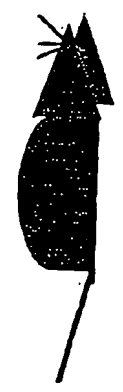
Immunization Groups:

Naïve

Vector Control

HIV-1 Gag

HIV-1 Gag/ IL15 constructs



*Combinations of 100µg IL15 Constructs, 50µg GAG,  
Each injection, intramuscular*

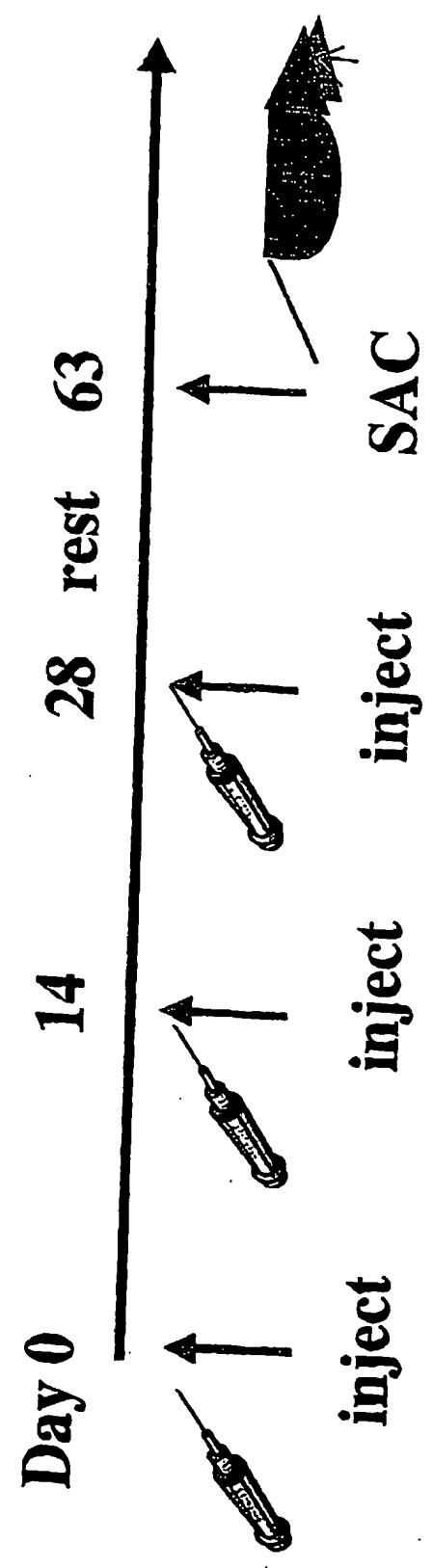
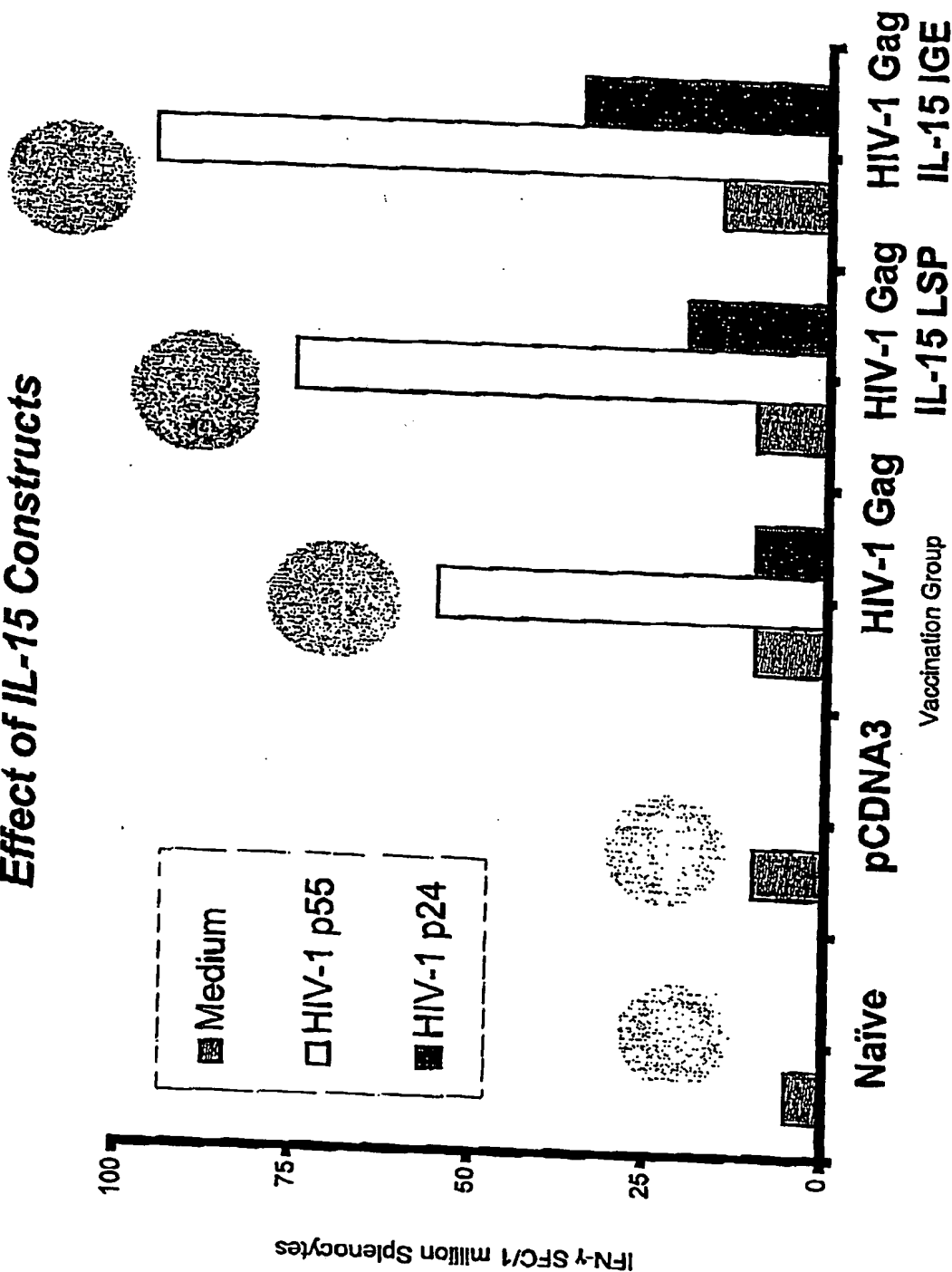


Figure 6

**Restimulation of antigen-specific IFN- $\gamma$  production 5 Weeks Following  
the 3rd immunization of HIV-1 Gag in Balb/C mice  
Effect of IL-15 Constructs**

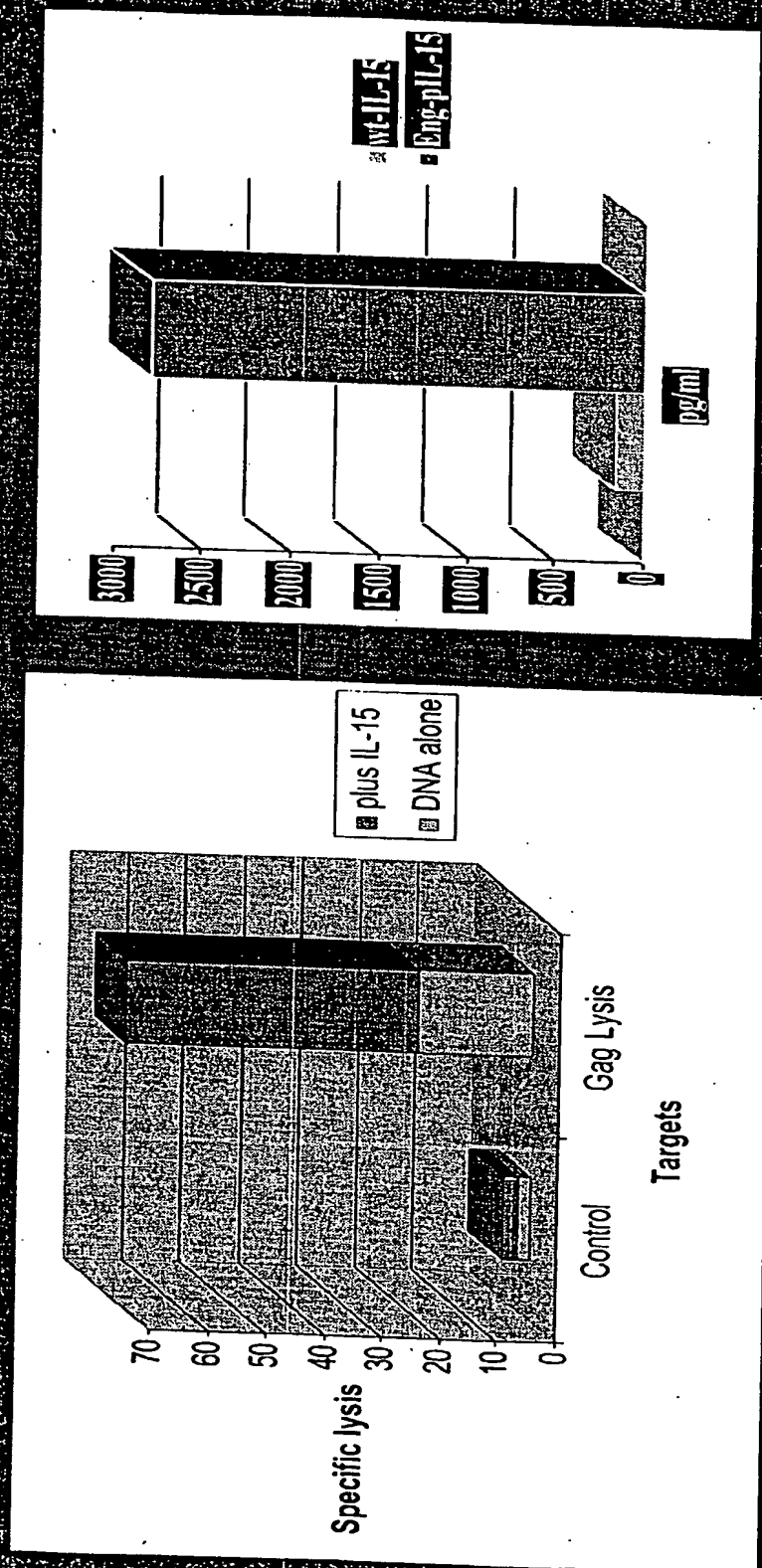


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# An Engineered IL-15 Plasmid Vaccine (Kozak, AUG's removed, UTR's removed & other-30- 50x better expression)

## Enhances CTL Response in Vivo

Mice were immunized with HIV-1 gag expressing DNA



Grabstein et al., (1994) Science 264:965-968, Bamford et al., (1996) PNAS 93:2897-2902,  
Bamford et al., (1998) J. Immunol 160:4418-4426, Kozak et al., (1991) J. Cell Biol. 115:887-903.

Figure 7



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